

U.S.S.N 10/014,731
Monforte
PRELIMINARY AMENDMENT

40. (Amended) The system of claim 35, wherein the one or more instruction sets comprise software for performing principle component analysis or difference analysis upon the plurality of data points.

REMARKS

Any fees that may be due in connection with this application throughout its pendency may be charged to Deposit Account No. 50-1213.

Claims 1-48 are pending. Claims 29, 34, and 36-40 are amended to correct minor typographical errors. Claims 25 and 34 are amended to correct claim dependency errors. Claim 1 is amended to add the inadvertently omitted conjunction "and" for grammatical clarity. Claim 13 is amended to add the inadvertently omitted words "in" and "contacting the sample with the at least one genetic package" for grammatical clarity. The amendment finds basis at page 4, lines 24-27, of the specification and finds basis in claim 1. Claim 24 is amended to add the inadvertently omitted word "component" for grammatical clarity. The amendment finds basis in claim 1. Claim 27 is amended to add the inadvertently omitted words "wherein" and "component" for grammatical clarity. The amendment finds basis in claim 1. No new matter has been added.

The specification is amended to correct obvious typographical and spelling errors and to produce grammatical clarity. The amendment to the paragraph on page 2, line 26, through page 3, line 2, of the specification adds the inadvertently omitted preposition "on" for grammatical clarity. The amendment to the paragraphs on page 25, line 27, through page 27, line 6, of the specification adds the inadvertently omitted verb "are" for grammatical clarity. The amendment to the paragraphs on page 36, line 9, through page 37, line 8, of the specification adds the inadvertently omitted verb "used" for grammatical clarity. The amendment to the paragraph on page 37, line 25, through page 38, line 17, of the specification adds the inadvertently omitted word "that" for grammatical clarity. No new matter has been added.

U.S.S.N 10/014,731
Monforte
PRELIMINARY AMENDMENT

The Abstract is amended to correct a minor typographical error. No new matter has been added.

Included as an attachment is a marked-up version of the specification paragraphs that are being amended, per 37 CFR §1.121.

* * *

Entry of this amendment and examination of the application are respectfully requested.

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: _____

Stephanie Seidman
Registration No. 33,779

Attorney Docket No. 24743-2308
Address all correspondence to:
Stephanie Seidman, Esq.
HELLER EHRMAN WHITE & McAULIFFE
4350 La Jolla Village Drive, 7th Floor
San Diego, California 92122-1246
Telephone: 858 450-8400
Facsimile: 858 587-5360
email:sseidman@HEWM.com



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Monforte
Serial No.: 10/014,731
Filed: December 11, 2001
Confirmation No.: 5775
For: *MULTIPLEXED PROTEIN EXPRESSION AND
ACTIVITY ASSAY*

Art Unit: 1743
Examiner: Unassigned

**ATTACHMENT TO THE PRELIMINARY AMENDMENT
MARKED UP PARAGRAPHS AND CLAIMS (37 CFR §1.121)**

IN THE SPECIFICATION:

Please amend the specification as follows:

Please amend the paragraph beginning on page 3, line 22, through page 4, line 2, as follows:

SUMMARY

Multiplexed assays for determining protein expression levels and activity that provide the above-noted features and many others are provided. The assays provided herein can simultaneously monitor hundreds of proteins within a sample. An exemplary assay employs display binding proteins, such as phage-displayed antibodies, to which [a] proteins of interest [proteins] bind. Each phage can contain a marker component. After binding to the protein or proteins of interest, the phage or the marker component within the phage, if necessary or desired, is amplified, and the marker component detected, such as with mass spectrometry. The quantity of marker component detected is directly related to the amount of protein in the sample.

Please amend the paragraph on page 4, lines 12-30, as follows:

Methods provided herein include the steps of contacting a sample, such as a blood or other body fluid or tissue sample that contains one or more

[polypeptide]polypeptides of interest, with at least one genetic package, such as a bacteriophage, a baculovirus, or a bacterium. Bacteriophages of interest include, but are not limited to, T4 phage, M13 phage, λ phage and any other phage [know] known to those of skill in the art. The genetic package is selected to display a polypeptide-binding component, such as an antibody on its surface. Other polypeptide-binding components, include, but are not limited to, antibody fragments, single chain antibody fragments, enzymes, biotin, avidin, streptavidin, ligands and receptors. The antibodies, antibody fragments or single chain antibody fragments generally contain one or more antigen recognition regions that bind to a target polypeptide. Contacting the sample with the genetic package includes contacting the sample with a plurality of bio-displayed polypeptide binding components that [are] can bind to one or more target [polypeptide]polypeptides in the sample. The plurality of bio-displayed polypeptide binding components can contain about 10^2 to about 10^{10} different polypeptide-binding components. For example, in a screening assay, 10^2 to about 10^5 different polypeptide-binding components can be used.

Please amend the paragraph on page 6, lines 21-28, as follows:

In another aspect, integrated systems for detecting one or more target [polypeptide]polypeptides in one or more samples are provided. The systems can include, for example, a plurality of bio-displayed polypeptide binding components and a solid support containing one or more target [polypeptide]polypeptides. The polypeptide binding components are designed or selected to bind to one or more of the target polypeptides. In addition, each polypeptide-binding component is associated with a different marker component, generally with a predetermined marker component.

Please amend the paragraph on page 8, lines 24-31, as follows:

As used herein, high-throughput screening (HTS) refers to processes that test a large number of samples, such as samples of test proteins or cells

U.S.S.N 10/014,731

Monforte

PRELIMINARY AMENDMENT ATTACHMENT

containing nucleic acids encoding the proteins of interest to identify structures of interest or [the]to identify test compounds that interact with the variant proteins or cells containing them. HTS operations are amenable to automation and can be computerized to handle sample preparation, assay procedures and the subsequent processing of large volumes of data.

Please amend the paragraph beginning on page 12, line 25, through page 13, line 29, as follows:

As used herein, an "antibody" refers to a polypeptide or protein substantially or partially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. An exemplary immunoglobulin or antibody structural unit includes a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 [kD]kDa) and one "heavy" chain (about 50-70 [kD]kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. Antibodies exist as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the (Fab')2 dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (see, *e.g.*, *Fundamental Immunology*, W.E. Paul, ed., Raven Press, N.Y. Fourth Edition (1998), for other antibody fragments known to those of skill in the art). While various antibody

PRELIMINARY AMENDMENT ATTACHMENT

fragments are defined in terms of the digestion of an intact antibody, one of skill in the art will appreciate that such Fab' fragments may be synthesized *de novo* either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized *de novo* using recombinant DNA methodologies. Antibodies also include single chain antibodies, including single chain Fv (scFv) antibodies in which a variable heavy and a variable light chain are joined together directly or through a linker, such as a peptide linker, to form a continuous polypeptide. [Thee]The antibodies, antibody fragments and single chain antibodies include, for example, an antigen recognition region, or a site that specifically recognizes and complexes with a specific antigen or target polypeptide.

Please amend the paragraph on page 20, lines 16-17, as follows:

As used herein, a combination refers to any association between two or more items. A combination can be packaged as a [kit]kit.

Please amend the paragraph beginning on page 22, line 26, through page 23, line 2, as follows:

As used herein, matrix or support particles or beads refers to support materials that are in the form of discrete particles. The particles have any shape and dimensions, but typically have at least one dimension that is 100 mm or less, 50 mm or less, 10 mm or less, 1 mm or less, 100 μm or less, 50 μm or less and typically have a size that is 100 mm^3 or less, 50 mm^3 or less, 10 mm^3 or less, and 1 mm^3 or less, 100 μm^3 or less and may be on order of cubic microns. Such particles are collectively called "beads."

Please amend the paragraphs beginning on page 23, line 23, through page 24, line 16, as follows:

B. Assays

Multiplexed assays for protein expression and activity are provided. Such assays can be used to understand the function of [a] complex systems that include a multitude of proteins and to understand the pathways and networks in which such proteins interact. Provided are assays for monitoring such complex systems by simultaneously monitoring the expression level and/or functional states of target proteins. Hundreds and thousands of proteins can be monitored simultaneously.

The assays simultaneously monitor the expression levels and functional state of a plurality of target proteins. The protein detection begins in a manner similar to a classic two-antibody sandwich assay. One binding moiety, such as an antibody, is attached to a solid phase support, such as [a] synthetic microspheres or "beads" or flat support, and captures a target protein. A second binding moiety, such as a second antibody, recognizes the captured target protein and binds to it, forming the two-binding moiety-target protein sandwich. Attached to the second binding moiety is a signal-generating element. In a standard ELISA, this element might be an enzyme, such as horse radish peroxidase (HRP). In the methods provided herein, the signal-generating element is a genetic package, such as a bacteriophage, that can infect and multiply within a host (the amplification component) and also code for the expression of a unique signature polypeptide (the detectable signal component) that is subsequently detected. In embodiments herein the signature polypeptide is detected and quantitated by mass spectrometry.

Please amend the paragraph on page 24, lines 23-29, as follows:

In one embodiment, a polypeptide binding component is displayed on a genetic package, such as a phage displayed antibody or binding [portio]portion thereof, and used to bind the target proteins. The genetic package contains a

predetermined marker component, such as a signature polypeptide or polynucleotide, that is detected and correlated to the amount of target protein bound by the polypeptide binding component on the genetic package.

Please amend the paragraphs beginning on page 25, line 27, through page 27, line 6, as follows:

1. Components of the methods

a. Samples

As noted, a sample generally contains one or more ~~[biomolecule]~~biomolecules. Samples can be from any source including biological materials, such ~~[a]~~as body fluids, tissues and organs, cells from prokaryotic or eukaryotic organisms. The cells can be plant or animal cells. Thus, cells include any cells known to those of skill in the art and ~~[included]~~include, but are not limited to, cells derived from a sample, such as a tissue sample, a body fluid, including blood, sweat, urine, synovial fluid and cerebral spinal fluid (CSF) samples, a tissue or organ sample, a cell lysate, or a sample from plurality of cultured cells. Such samples generally contain and/or express polypeptides and/or proteins of interest. Alternatively, the samples are mixtures, such as isolated or recombinant polypeptides for which an assay is desired, such as to determine functional state. A sample can contain about 3 to about 100 or about 500 target polypeptides or about 50 to about 10,000 target polypeptides or about 100 to 5000 target polypeptides or more up to any desired number, including, for example, all proteins in a cell or tissue or cell culture lysate. In some embodiments, the target polypeptides contain biotin, avidin, lectin, a small organic molecule or other moiety to aid in isolation, immobilization and/or detection.

The methods and integrated systems provided herein optionally are used to analyze any target polypeptide or mixture of target polypeptides. The target polypeptides are generally in a sample, which is assayed using genetic packages displaying polypeptide binding components as described herein. The target

proteins bind to the bio-display binding moieties, such as bio-displayed polypeptides, such as antibodies or fragments thereof.

Each bio-displayed polypeptide binding component is associated with a predetermined marker, which is used as a signal component to detect the target polypeptide. The samples are contacted with the bio-displayed components for binding to occur. For example, the target polypeptides of the sample are bound to a solid [support]support, and the bio-displayed polypeptide binding components are applied to the solid support. After removal of any unbound polypeptide binding component, the genetic package or bio-display component is [amplified]amplified, or the predetermined marker component within the genetic package is [amplified]amplified, and the marker components are detected and optionally quantitated. The presence of and amount of each marker component correlates to the presence of and amount of the target polypeptide to which the genetic package associated with the marker component was bound, such as, for example, via a polypeptide binding component. Each of these steps and components is described in more detail below.

Please amend the paragraph beginning on page 31, line 17, through page 32, line 2, as follows:

e. Phage-Displayed Antibodies

1) Phage, viruses and bacteria for displaying binding polypeptides

Phage, viruses, bacteria and other such manipulable hosts and vectors (referred to as biological particles) can be modified to express selected antigens (peptides or polypeptides) on their surfaces by, for example, inserting DNA encoding the antigen into the host or vector genome, at a site such as in the DNA encoding the coat protein, such that upon expression the antigen (peptide or polypeptide) is presented on the surface of the virus, phage or bacterial host. Libraries of such particles that express diverse or families of proteins on their

surfaces can be prepared and the resulting library is screened with [a] target proteins [(i.e. receptors or ligands).](i.e. receptors or ligands). Those viruses with the highest affinity for the targeted antigen (receptor or ligand) can be selected (see, e.g., U.S. Patent Nos. 5,403,484, 5,395,750, 5,382,513, 5,316,922, 5,288,622, 5,223,409, 5,223,408 and 5,348,867).

Please amend the paragraphs beginning on page 36, line 9, through page 37, line 8, as follows:

Virtually any phage-display technique or library [is] can be used in the signal detection methods used herein. For example, the method is easily adapted using methods well known to those of skill in the art to work with a variety of display systems such as *E. coli* flagellin fusion displays or T7 phage-display. For detection, the affinity recognition of a target protein is linked to or associated with a predetermined marker component, such as signature polypeptide or nucleic acid molecule encoding a signature peptide.

2) Phage and other display methods for generating and displaying moieties that specifically bind to target polypeptides

In this embodiment, antibodies, fragments thereof or other such binding moieties are employed for specific binding to target polypeptides. This method provides a high level of flexibility and selectivity because antibodies can be selected for virtually any target or mixture of target polypeptides. For example, a plurality of bio-displayed components displaying selective antibodies for about 100 different target proteins can be used. In addition, antibodies can be used to identify particular functional forms of a given target protein, including pre-and post- processed forms, active and inactive forms, presence of modifications, such as for example, phosphorylation and glycosylation, conformational changes, and the presence of protein-ligand interactions. Multiple antibodies are optionally used, such as by displaying them on a genetic package(s) to provide a detailed functional and structural map of a given target polypeptide, such as, for example, by epitope mapping.

U.S.S.N 10/014,731
Monforte
PRELIMINARY AMENDMENT ATTACHMENT

In one exemplary embodiment, the methods are based on immunological detection using antibody-antigen recognition of the target polypeptides and can be practiced using phage-displayed antibodies. The target polypeptide is the antigen in such a [system]system, and an antibody or fragment thereof that recognizes and binds to the target polypeptide is used as the polypeptide binding component.

Please amend the paragraph beginning on page 37, line 25, through page 38, line 17, as follows:

The antigenic targets, such as the target polypeptides obtained, for example, from cells or tissues, that are used to select antibodies can be purified [and]in intact forms of the target protein. Isolation of native, intact proteins can be laborious and quite frequently, an antibody is actually used to make purification feasible. The methods herein circumvent this requirement for purified native target polypeptides while still selecting specific antibodies against the desired antigen, e.g., a target polypeptide. The methods use synthetic polypeptides and recombinant expression systems in which proteins are optionally over-expressed within a host cell. Using standard cloning techniques, synthetic genes derived from complete or partial cDNA sequences are inserted into an expression vector creating an expression cassette (see, *e.g.*, Ausubel *et al.* (1987) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York) that, upon introduction into an appropriate host, drives the high level expression of the gene of interest. Isolation of recombinant, over-expressed proteins is often made possible by the fact that the protein of interest is expressed as a chimeric fusion to another protein or peptide sequence tag that facilitates direct isolation (see, *e.g.*, Ausubel *et al.* (1987) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York; Promega, Inc.'s "PINPOINT™ System") The PINPOINT™ System for direct isolation allows for the expression of a target protein as a biotinylated conjugate. These biotinylated proteins can be

directly captured using a streptavidin-modified substrate and then directly used in panning experiments as described above.

Please amend the paragraph on page 44, lines 1-12, as follows:

As described above with respect to signature polypeptides, the signature polynucleotides do not have to have, and generally do not have, any sequence relationship to the target polypeptide. The signature polynucleotide is a nucleic acid molecule or fragment that is contained within the bio-display component, such as integrated into a phage genome. Each signature polynucleotide is associated with or correlated with a specific polypeptide-binding component and their identity is known or can be known. They are used as signature markers that [are] can be directly detected or amplified prior to detection. Amplification can be effected using PCR, host infection, electroporation, or the like. Detection of signature polynucleotides or the encoded protein can be correlated with the amount of target polypeptide in a sample.

Please amend the paragraph on page 48, lines 12-23, as follows:

b. Exemplary Amplification Techniques

After contacting and binding one or more polypeptide binding component displayed on a genetic package to one or more target polypeptide in a sample, the bound genetic packages are released or eluted from the target [polypeptide..]polypeptide. For example, they are removed from a solid substrate to which the target polypeptides were bound, such as via an antibody. The genetic packages, if desired, can then be amplified. Amplification can be accomplished in bacterial host, for example, to produce one or more amplified genetic packages. In one embodiment, a predetermined polypeptide marker in the genetic package is over expressed in the bacterial host. Alternatively, a predetermined polynucleotide marker component within the genetic package is amplified.

Please amend the paragraph on page 54, lines 12-23, as follows:

For example, mass spectrometry is among the methods provided herein for detecting proteins. Mass spectrometry can be coupled to protein isolation or segregation methods, such as high performance liquid chromatograph (HPLC), and used to analyze complex mixtures of proteins (see, *e.g.*, Opiteck *et al.* (1998) *Anal. Biochem.* 258:349-61; and Woo *et al.* (1994) *Clin. Lab. Med.* 14:459-71 for mass spectrometric protein analysis methods). Direct mass measurements can be used to monitor protein modifications, such as phosphorylation or glycosylation. Because of the complexity of the samples to be [analyzed]analyzed, direct visualization methods using mass spectrometry are relatively [slow]slow, and the data [is]are challenging to analyze (see, *e.g.*, Arnott *et al.* (1998) *Anal. Biochem.* 258:1-18; and Opiteck *et al.* (1998) *Anal. Biochem.* 258:349-61).

Please amend the paragraph on page 65, lines 12-30, as follows:

A high throughput, scanning MALDI-TOF mass spectrometer provides one example of a detection system that is easily integrated into the multiplexed systems. Mass spectrometry systems that process large numbers of samples without the need for exchanging sample plates are known and available. Multiple sample trays of standard microtiter plate dimensions are processed in an uninterrupted fashion. Sample densities start at about 384 samples per plate, up to about 1,536 samples and higher. Since mass spectrometers generally scan through and analyze samples serially, the overall analysis time is a multiple of the per-sample analysis time. Analysis of small peptides is straightforward and per-sample analysis time [is] can average about 1 to about 3 seconds per sample, providing an expected throughput of about 1,200 to about 3,600 samples per system, per hour (independent of multiplexing). When multiplexing is performed as described above, each sample contains a mixture of signature polypeptides, therefore providing a higher throughput for signature polypeptide detection. For example, if each sample contains about 20

U.S.S.N 10/014,731
Monforte
PRELIMINARY AMENDMENT ATTACHMENT

polypeptides, then the multiplexed assay described above detects about 24,000 to about 72,000 polypeptides in about an hour, thereby providing a high throughput assay system.

Please amend the paragraph beginning on page 66, line 7, through page 67, line 2, as follows:

After detection of the predetermined marker components, such as the amplified signature polynucleotides, an analysis module, such as a computer, in operational communication with the detection system is used for data analysis, such as correlating the amount of each target polypeptide in the sample with the amount of predetermined marker component detected. For example, the analysis module optionally calculates a ratio of a first marker component to a second marker component and optionally correlates that ratio to the ratio of a first and second target polypeptide in the sample that was assayed. The ratios are determined from the data obtained by the detection system, which generates a plurality of data points based, for example, on the amount and/or identity of each marker component. The data points are used in the analysis module, in which a computer or computer readable medium, containing one or more instruction set for organizing data points into a database, compiles the data points into a database containing a profile for each sample or each target polypeptide in a sample. The profiles can identify an expression level and a functional state for each target polypeptide in the sample. The instructions sets used to compile such profiles can include software for generating a graphical representation of the amount of each polypeptide. In addition, the instruction sets associated with the analysis module optionally include software for performing statistical analysis, such as, for example, multivariate analysis, principle component analysis, or difference analysis, on the [on the] plurality of data points. In addition, the software and the instruction sets [can] can produce an output file embodied in a computer readable medium, which output file can include the profiles described above.

U.S.S.N 10/014,731

Monforte

PRELIMINARY AMENDMENT ATTACHMENT

IN THE ABSTRACT:

Please amend the abstract as follows:

Please amend the paragraph on page 74, lines 1-6, as follows:

ABSTRACT

A system [is] for analyzing expression levels and activity of a plurality of proteins is provided. A bio-displayed polypeptide binding component associated with a predetermined marker is used to bind the proteins of interest. The predetermined marker components are then amplified and detected in a high throughput manner.

IN THE CLAIMS:

Please amend claims 1, 13, 24, 25, 27, 29, 34, and 36-40 as follows:

1. (Amended) A method of detecting one or more polypeptides in a sample, the method comprising:

a) contacting the sample with at least one genetic package that displays a polypeptide-binding component, wherein:

the genetic package comprises a predetermined marker component; and

the polypeptide binding component specifically binds to at least one of the polypeptides;

b) amplifying the genetic package, resulting in an amplified genetic package, or amplifying the marker component in the genetic package; and

c) detecting the marker component, wherein the presence of the marker component indicates the presence of the one or more polypeptides.

13. (Amended) The method of claim 1, wherein in step (a) contacting the sample with the at least one genetic package comprises contacting the sample with a plurality of bio-displayed polypeptide binding components.

24. (Amended) The method of claim 1, wherein the predetermined marker component comprises one or more signature polypeptides.

25. (Amended) The method of claim [23]24, wherein at least one of the one or more signature [polypeptide]polypeptides is derived from hemoglobin.

27. (Amended) The method of claim 1, wherein the predetermined marker component comprises a nucleic acid fragment.

29. (Amended) An integrated system for detecting one or more [polypeptide]polypeptides in one or more [sample]samples, the system comprising:

- a) a solid support comprising the one or more [polypeptide]polypeptides;
- b) a plurality of bio-displayed polypeptide binding components that bind to one or more of the one or more polypeptides, and are each associated with a different marker component;
- c) an assay module for amplifying or expressing the marker component;
- d) a detection module for receiving the marker component or a derivative thereof, wherein the detection system detects one or more different marker [component]components and determines an amount of the one or more different marker [component]components; and,
- e) an analyzing module in operational communication with the detection system that comprises a computer or computer readable medium comprising one or more instruction [set]sets for correlating the amount of the one or more different marker [component]components with the one or more [polypeptide]polypeptides.

34. (Amended) The system of [29]33, wherein the analyzing module correlates the ratio to a ratio of at least a first polypeptide to at least a second polypeptide in the one or more [sample]samples.

36. (Amended) The system of claim 35, wherein the computer or computer readable medium comprises an instruction set for organizing the data

U.S.S.N 10/014,731

Monforte

PRELIMINARY AMENDMENT ATTACHMENT

points into a database that comprises a profile for the one or more
[sample]samples.

37. (Amended) The system of claim 36, wherein the profile for the one or more [sample]samples identifies an expression level of at least one of the one or more [polypeptide]polypeptides in the sample and a functional state of at least one of the one or more [polypeptide]polypeptides in the sample.

38. (Amended) The system of claim 29, wherein the one or more instruction [set]sets [comprises]comprise software for generating a graphical representation of the amount of the one or more polypeptides.

39. (Amended) The system of claim 35, wherein the one or more instruction [set]sets [comprises]comprise software for performing multivariate analysis for the plurality of data points.

40. (Amended) The system of claim 35, wherein the one or more instruction [set]sets [comprises]comprise software for performing principle component analysis or difference analysis upon the plurality of data points.